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Biodecolorization and biodegradation potential of recalcitrant triphenylmethane dyes by *Coriolopsis* sp. isolated from compost

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ABSTRACT

Triphenylmethane dyes (TPM) are recalcitrant colorants brought into the environment. In this study, a lesser-known white rot fungus *Coriolopsis* sp. (1c3), isolated from compost of Empty Fruit Bunch (EFB) of oil palm, was explored for its decolorization potential of TPM dyes. The isolate 1c3 demonstrated good decolorization efficiencies in the treatment of Crystal Violet (CV; 100 mg l^{-1}), Methyl Violet (MV; 100 mg l^{-1}) and Cotton Blue (CB; 50 mg^{-1}), with 94%, 97% and 91%, within 7, 7 and 1 day(s), respectively. Malachite Green (MG; 100 mg l^{-1}) was the most recalcitrant dye, with 52% decolorization after 9 days. Dye removal by 1c3 was presumably via biosorption, whereby the process was determined to be influenced by fungal biomass, initial dye concentrations and oxygen requirements. Biodegradation was also a likely mechanism responsible for dye removal by 1c3, occurred as indicated by the reduction of dye spectra peaks. Detection of laccase, lignin peroxidase and NADH-DCIP reductase activities further substantiate the possible occurrence of biodegradation of TPM dyes by 1c3.

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1. Introduction

Triphenylmethane (TPM) dyes are synthetic, aromatic colorants used extensively in various textiles, paper, leather, and pharmaceutical industries (Daneshvar et al., 2007). Among the many types of TPM dyes, Crystal Violet (CV), Methyl Violet (MV), Malachite Green (MG) and Cotton Blue (CB) are extensively used resulting in 10–15% of unused dye released into the environment annually (Shedbalkar et al., 2008; Yang et al., 2009). TPM dyes are recalcitrant to degradation due to their synthetic nature and complex aromatic structures (Daneshvar et al., 2007). Their persistence in the environment leads to health and ecological concerns due to their toxic, mutagenic and carcinogenic nature (Jadhav and Govindwar, 2006).

Conventional approaches to remove TPM dyes include application of activated carbon, membrane filters, and chemical coagulation and flocculation (Saratale et al., 2006). These methods are generally effective, but are limited by steep investment costs and generation of toxic sludge (Shedbalkar et al., 2008). Bioremediation is implemented as an alternative as this approach has many benefits to the environment (Shedbalkar et al., 2008). Bioremediation of dyes can be achieved via biosorption or biodegradation. In biosorption, dye molecules bind to the surface of the biomass, while in biodegradation the enzymes are responsible to degrade large toxic dye molecules into simpler and less toxic compounds (Jadhav and Govindwar, 2006).

Historically, dye removal is predominantly performed by typical white rot fungi such as *Phanerochaete chrysosporium*, *Trametes versicolor*, *Pleurotus ostreatus* and *Bjerkandera adusta*; attributed to the role of their extracellular non-specific ligninolytic enzymes (Liu et al., 2004; Salame et al., 2012). These enzymes include laccase (Lac), lignin peroxidase (LiP) and manganese peroxidase (MnP) (Nishiya and Yamamoto, 2007; Zhuo et al., 2011; Anastasi et al., 2011). Fungal species capable of dye removal have been reported to produce at least one of these essential enzymes (Casas et al., 2009). In recent years, other fungal species have emerged with reports in removing TPM dyes. *Aspergillus fumigatus* and *Fusarium solani* (Martius) Sacchardo are two examples where biosorption of Acid Violet 49, CV and MG have been demonstrated (Abedin, 2008; Chaudhry et al., 2013). For biodegradation, only *Aspergillus* sp. has been established to biodegrade MG and MV (Saratale et al., 2006; Kumar et al., 2011, 2012).

In this study, the dye removal and biodegradation potential of a lesser-known white rot fungus, *Coriolopsis* sp. (1c3) was explored. Several species of this genus; *C. rigida*, *Coriolopsis gallica*, *Coriolopsis polyzona* and *C. byrsina*, have been reported as laccase producers able to decolorize anthraquinone, azo, heterocyclic and metal

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textile dyes (Sanchez-Lopez et al., 2008; Gomes et al., 2009; Chairin et al., 2013; Daâssi et al., 2014). Nevertheless, their potential to decolorize TPM dyes remains to be explored. Isolate 1c3 was recovered from the EFB compost of oil palm and was the isolate of interest as it had successfully decolorized the azo dye Remazol Brilliant Blue R (RBBR) with 70% decolorization efficiency (Peh et al., 2011). The isolate 1c3 was tested on four different TPM dyes (CV, MV, MG, CB). The optimum conditions for dye decolorization (presumably via biosorption) under the influence of fungal biomass, initial dye concentration and oxygen requirement were established. The biodegradation potential of 1c3 was detected from evaluations on the change of the absorption spectra, and the quantification of enzymatic activities (laccase, lignin peroxidase and NADH-DCIP reductase activities). This paper reports the results from this study with aims to provide information on the use of 1c3 for the removal of TPM dyes in wastewater.

2. Methods

2.1. Culture establishment and biomass generation

Isolate 1c3 was isolated by Peh et al. (2011) from Empty Fruit Bunch (EFB) compost of oil palm (*Elaeis guineensis*). The fungal isolate was maintained on Potato Dextrose Agar (PDA, Merck) at room temperature (25 ± 2 °C) and sub-cultured periodically. To generate biomass, five mycelial plugs of 1c3 were inoculated into 100 ml of Potato Dextrose Broth (PDB, Difco™, Le Pont de Claix, France) and incubated for 7 days (25 ± 2 °C). The fungal biomass was homogenized with a hand-held homogenizer (LabGEN 125, Cole-Palmer, USA), filtered through Whatman filter paper No. 1, washed with sterile distilled water and weighed to 2.0 ± 0.1 g (fresh weight). This freshly-prepared biomass is used for dye decolorization experiments.

2.2. Dye decolorization tests

Each TPM dye (CV, MV, MG and CB) was dissolved in 100 ml autoclaved MilliQ water (18.2 MQ; Sartorius, Malaysia) to a concentration of 100 mg l^{-1} (except CB to a concentration of 50 mg l^{-1}). To the dye solutions, 2.0 ± 0.1 g of the freshly prepared fungal biomass was introduced and the mixture incubated with agitation (150 rpm, 30 ± 2 °C) (MaxQ 6000, Thermo Scientific, Iowa, USA) for 14 days. Untreated (non-inoculated) dye solutions were designated as negative controls. At every 24 h-interval for the next 14 days, a 2 ml of aliquot was withdrawn, centrifuged (10,000 rpm, 10 min) (Microfuge 22R centrifuge, Beckman Coulter, Germany) and the supernatant collected for absorbance analysis. Each dye solution was measured at its respective wavelength (CV at 590 nm, MV at 584 nm, MG at 617 nm, CB at 599 nm) using a Tecan Infinite M200 plate reader. The dye removal potential by isolate 1c3 is expressed as decolorization efficiency (DE, %) (Parshetti et al., 2006) as follows:

$$\mathrm{DE} = \frac{A_i - A_o}{A_o} \times 100$$

where A_i and A_o represent the initial and observed absorbance post-treatment with 1c3, respectively.

2.3. Optimization of conditions for dye decolorization

Dye decolorization by isolate 1c3 under the influence of fungal biomass, initial dye concentration and oxygen requirement were investigated. The optimum biomass was determined by treating 100 ml of dye solutions with fungal biomass at various weights of 1.0, 2.0, 4.0, 6.0 and 8.0 ± 0.1 g. The initial dye concentrations were kept constantly at 50 mg l^{-1} for CB and 100 mg l^{-1} for CV, MV and MG, as with other experimental conditions outlined in Section 2.2. The influence of initial dye concentrations was tested using 50, 100 and 200 mg l^{-1} of each dye while all other factors remained constant. For the oxygen requirement test, 100 ml of dye solutions were first inoculated with 2.0 ± 0.1 g (fresh weight) of fungal biomass, and overlaid with 5 ml of paraffin oil (filter-sterilized with 0.45 μm mixed cellulose ester syringe filter (Jet Biofil)) prior to incubation as standing static cultures (anaerobic set). A separate flask was prepared with the exclusion of paraffin overlay, and incubated with agitation to allow dye decolorization under aerobic conditions.

2.4. Ultraviolet–visible (UV–vis) spectral analysis to detect biodegradation potential

UV–vis spectral analysis was used to detect the possible occurrence of biodegradation, particularly when change in peaks between control and treated dye samples were observed (Kalpana et al., 2012). The experiment was initiated by inoculating 2.0 ± 0.1 g of harvested fungal biomass in 100 ml of respective dye solutions and incubation followed (150 rpm, 30 ± 2 °C). Non-inoculated dye solutions were included as negative controls. For the next 14 days, 2 ml aliquots were withdrawn at every 24-h interval, centrifuged (10,000 rpm, 10 min) and the supernatant was measured by means of UV–vis between the wavelengths of 300–800 nm, using a Tecan Infinite M200 plate reader, to detect absorption peaks of each TPM dye (Parshetti et al., 2011). Spectra peaks for dyes with and without treatment of 1c3 were plotted and compared.

2.5. Enzyme assays to detect biodegradation potential

Dye solutions (50 mg l^{-1} dye concentrations) were treated with 1c3 according to optimum conditions derived from the results. Approximately 2.0 ± 0.1 g of 1c3 were introduced into CV and MV solutions, 4.0 ± 0.1 g in CB and 8.0 ± 0.1 g in MG solutions. Treated CV, MV and CB solutions were incubated with agitation, while MG was overlay with paraffin oil. The control was prepared by inoculating PDB with 5 mycelial plugs and incubated with agitation (150 rpm, 30 ± 2 °C). For both treated and control sets, the enzymatic activities were determined at every 1 h interval for the first 6 h, gradually increasing to 18, 24, 48, 72, 96, 120, 144, 168, 192, 216, 240, 264, 288, 312 and 336 h, when enzyme levels were discovered to increase after 6 h of incubation. The three key enzymes assayed were laccase (Lac), lignin peroxidase (LiP) and NADH-dependent 2, 6-dichlorophenolindophenol (NADH-DCIP) reductase.

To perform the laccase (Lac) assay, 300 μl of supernatant was introduced to 2.3 ml reaction mixture (1400 μl distilled water, 600 μl of 0.1 M pH 5 sodium acetate buffer, 300 μl of 5 mM ABTS) and incubated at 30 ± 2 °C (Wisebath, WB-15; Daihan Scientific, Seoul, Korea) for 2 min. Hydrogen peroxide (300 μl of 1 mM H_2O_2) was added and the change in absorbance was immediately measured at 1 min intervals for 15 min. The calorimetric change due to oxidation of ABTS was detected at 420 nm (molar extinction coefficient, ε_{420} = 36,000 M^{-1} cm^{-1}) using a Tecan Infinite M200 plate reader (Bourbounnais et al., 1995). Lac activity was compared to a standard curve constructed from 0.0 to 0.01 U ml^{-1} concentrations of commercially purified laccase of *Trametes versicolor* (Sigma). One unit of laccase activity (U) is expressed as the activity of laccase required to catalyze the conversion of 1 μmole of ABTS per minute (Bourbounnais et al., 1995).

To quantify lignin peroxidase (LiP), 60 μl of supernatant were added to 2.85 ml of the reaction mixture (1890 μl distilled water, 600 μl of 0.3 M citrate-0.4 M phosphate buffer with pH 4.5, 300 μl of 8 mM veratryl alcohol) and incubated as previously described. Hydrogen peroxide (150 μl of 5 mM H_2O_2) was added and the change in absorbance as a result of the oxidation of veratryl alcohol

to veratryl aldehyde, was read at 310 nm ($\varepsilon_{310} = 9300\ M^{-1}\ cm^{-1}$) at 1 min intervals for 15 min (Takamiya et al., 2008). LiP activity was compared against a standard curve (0.00–0.24 U ml^{-1}) of commercially purified LiP (Sigma), with one unit of LiP expressed as the activity of LiP required to catalyse the conversion of 1 μmole of veratryl alcohol per minute (Takamiya et al., 2008).

For NADH-DCIP reductase assay, 100 μl of supernatant was added into 4 ml of reaction mixture (50 μM of DCIP, 50 μM of NADH in 50 mM potassium phosphate buffer at pH 7.4). The change in absorbance as a result of DCIP reduction (extinction coefficient 19 $mM^{-1}\ cm^{-1}$) (Du et al., 2011) within the first minute was immediately read at 590 nm. The NADH-DCIP reductase activity (μmol $min^{-1}\ ml^{-1}$) was then calculated according to Dionisio-Sese and Tobita (1998):

$$\frac{\left(\frac{\Delta A}{\min_s} - \frac{\Delta A}{\min_b}\right) xV}{\varepsilon x d x v}$$

where $\Delta A/\min_s$ indicates change in absorbance per min for the sample, $\Delta A/\min_b$ is the change in absorbance per min for the blank; V is the total volume of the reaction mixture (ml); ε is the molar extinction coefficient ($mM^{-1}\ cm^{-1}$); *d* is the pathlength (cm); and *v* is the volume of enzyme solution (ml).

2.6. Enumeration of viable fungal cells

Viable cell count was performed on fungal biomass on the first and last day of the decolorization experiments (Section 2.2) and optimization studies (Section 2.3). Briefly, from the inoculated dye solutions, serial dilution to 10^{-6} was performed and spread-plated onto PDA. The plates were incubated at room temperature (25 ± 2 °C) for 5 days, in which the numbers of colonies formed were then enumerated (CFU ml^{-1}). The percentage of viable cells (%) was calculated from López-Amorós et al. (1995) as follows:

$$\frac{\text{live cell count on the last day of experiment}}{\text{live cell count on the first day of}} \times 100$$

2.7. Identification of isolate 1c3

Slide cultures of 1c3 were prepared on PDA blocks according to Harris (1986). After incubation at room temperature (25 ± 2 °C) for 8 days, staining with lactophenol cotton blue was performed. Microscopic examinations of the isolate were captured by a DP21 digital camera attached to a microscope (Olympus Corporation). Sequencing was also performed to identify the isolate. Broth cultures were established in PDB (incubated for 7 days, 25 ± 2 °C) and the fresh mycelia (30 mg wet weight) used for genomic DNA extraction with the GF-1 Plant DNA Extraction Kit (Vivantis Technologies, California, USA). Primer pairs ITS 5 (5′-GGAAGTAAAAGTCGTAACAAGG-3′) and ITS 4 (5′-TCCTCCGCTTATTGATATGC-3′) were used to amplify and sequence the internal transcribed spacer (ITS1, 5.8S rRNA, ITS2) gene region (White et al., 1990). PCR amplifications were performed in a 50 μl reaction mixture consisting of 10–15 ng of genomic DNA, 25 pmol of each primer, 25 μl GoTaq Green Master Mix 2× (Promega, Malaysia) and top up with nuclease-free water (Promega, Malaysia). Conditions for amplification process on a MyCycler Thermo Cycler (Bio-Rad) includes: initial denaturation at 95 °C for 10 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 1 min and a final extension at 72 °C for 10 min. PCR products obtained were purified with MEGAquick-spin™ Total Fragment DNA Purification Kit (iNtRON Biotechnology, Korea) and outsourced to 1st Base (Malaysia) for DNA sequencing. The sequence results obtained were compared to those in the database from the National Center for Biotechnology Information (NCBI) using BLAST (http://www.ncbi.nlm.nih.gov/). The sequence wa uploaded and deposited in NCBI and the accession number obtained. A phylogenetic tree was also generated on Phylogeny.ft platform (http://www.phylogeny.fr/) using default parameters of MUSCLE to align the sequences, PhyML to infer phylogenetic tree, TreeDyn to render the tree and the approximate likelihood-ratio test to estimate the branch support (Dereeper et al., 2008).

2.8. Statistical analysis

Triplicates were prepared for all experiments. The data obtained was analyzed with Analysis of Variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) version 20.0. Means were compared with Tukey–Kramer multiple comparison test (Honestly Significant Difference, $P < 0.05$), or a paired t-test ($P < 0.05$ for paired-comparisons) if relevant.

3. Results and discussion

3.1. Dye removal potential of 1c3

Isolate 1c3 demonstrated strong decolorization efficiency (DE, %) towards MV, CV, and CB with 97%, 95% and 91%, respectively. MG was more recalcitrant with only 52% DE achieved. Rapid decolorization was also observed for CB with maximum DE (%) attained at day 1, followed by MV (day 7), CV (day 7) and lastly MG (day 9). Decolorization efficiency may be attributed to the molecular structures of the dye, particularly the electron distribution, steric factors and charge density of the dye molecules (Liu et al., 2004). These factors affect the interaction of the biosorbent (1c3) with the dyes. It was reported that the presence of electron-withdrawing groups (-SO_3^- groups) on CB renders the molecule to rapid degradation, while other dye molecules (CV, MV, MG) with more electron-donating groups (-CH_3 groups) are degraded less rapidly (Hsueh et al., 2009; Chen et al., 2010).

3.2. Biodegradation potential

The change in peak patterns for TPM dyes based on adsorption spectra (300–800 nm) strongly suggest the biodegradation potential of 1c3 for all four dyes. For CV, MV, MG and CB, absorbance peaks were moved from 590, 584, 617 and 599 nm to 560, 570, 610 and 0 nm, respectively, after decolorization with 1c3. Biodegradation of CB was the most prominent as absorbance peaks disappeared after decolorization by 1c3 (Fig. 1). This is a typical result that strongly suggests the occurrence of biodegradation as a mechanism to remove the dyes as the breakdown of the chromophoric groups would lead to movement, reduction or disappearance of absorbance peaks. This could be the first report to document the biodegradation potential of a lesser-known white rot fungi *Coriolopsis* sp. on CV, MV, MG and CB, joining the list of other isolates such as *Bacillus* sp. (Ayed et al., 2009), *Aspergillus* sp. (Kumar et al., 2011), *Saccharomyces cerevisiae* MTCC 463 (Jadhav and Govindwar, 2006) and *Penicillium ochrochloron* MTCC 517 (Shedbalkar et al., 2008) in degrading the four TPM dyes, respectively.

3.3. Optimization of decolorization conditions for 1c3

3.3.1. Influence of biomass used

The amount of biomass used was observed to have an effect on the decolorization efficiency of 1c3 for all four TPM dyes tested. Results indicated that the minimum biomass required for decolorization activity was 2 g as the increase in biomass from 1 to 2 g

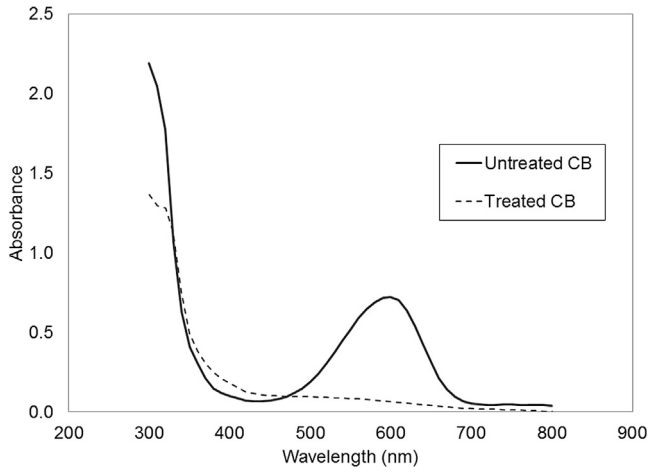

Fig. 1. Exemplary figure of the UV–vis spectrum analysis of Cotton Blue (CB, 50 mg l^{-1}) before (untreated, ——) and after decolorization (treated, – –) by 2 g of 1c3 at 30 ± 2 °C. (UV-spectrum analysis for other dyes are provided as supplementary material).

led to significantly greater DE for all dyes (except CB) (Fig. 2). Subsequent increase to 4, 6 and 8 g of biomass did not enhance DE for CV, with DE values ranging from 71 to 87% (Fig. 2). By contrast, increase in biomass from 6 to 8 g improved DE on MV and CB (86% and 79% at 8 g, respectively) while 8 g of biomass was able to generate significant decolorization activity (70%) on MG (Fig. 2). The enhance DE as a consequence of higher biomass used is also observed by Radha et al. (2005; Jadhav and Govindwar (2006); Saratale et al. (2006); Parshetti et al. (2006) and is primarily attributed to more fungal cells available for biosorption and to secrete more extracellular enzymes responsible for biodegradation (Abedin, 2008).

The biomass also influenced the rate of decolorization to achieve maximum DE (%). When 2 g biomass of 1c3 was applied, maximum DE for CV was attained by 168 h (Fig. 3). By contrast, when 4 g biomass was used, a faster decolorization was achieved by 72 h. For CB, MV and MG, higher biomass allowed maximum DE to be achieved by 18 h with the use of 4, 8 and 8 g of biomass, respectively. In fact, 8 g of biomass successfully attained maximum DE within 18 h for all dyes tested. The use of biomass is also dependent on the dye

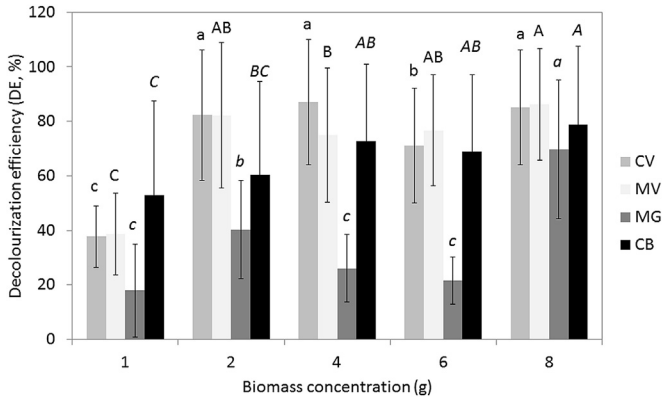

Fig. 2. Influence of biomass (1, 2,4, 6 and 8 g) of 1c3 on the decolorization activities on Crystal Violet (CV), Methyl Violet (MV), Malachite Green (MG) and Cotton Blue (CB). Means with the same letters and captions are not significantly different at Honestly Significant Difference (HSD $_{(0.05)}$). Mean comparisons are made for means with the same fonts. For example, means designated "A" compared to another mean with "A" Tukey grouping is not significantly different, while "A" compared to "B" and "C" is significantly different. Similarly, means with "a" is not significantly different compared to "a" but is for "b" and "c". Bars indicate standard deviation of mean (±SD).

concentrations. From the results here, 2 g is considered the optimum biomass to remove 50–100 mg l^{-1} of TPM dyes although 8 g allows decolorization to occur more rapidly, as generation of more biomass would incur additional cost (Fig. 3). The use of a higher amount of biomass would only be considered should concentrations of dyes are higher than 100 mg l^{-1} and when rapid decolorization is necessary. This has been proven by Saratale et al. (2006) who demonstrated that 200 and 500 mg $l^{-1}$of TPM dyes were efficiently decolourized within 24 h by 10 g (wet weight) of *Aspergillus ochraceus*. By contrast, 0.5 g of *P. ochrochloron* was sufficient to decolourize (93% DE) 50 mg L^{-1} of CB within 2.5 h (Shedbalkar et al., 2008).

3.3.2. Influence of initial dye concentrations

The DE for isolate 1c3 on TPM dyes appeared to be better at lower initial dye concentrations (50, 100 mg l^{-1}) compared to higher concentration (200 mg l^{-1}). At 200 mg l^{-1} initial dye concentrations for CV, MV, MG and CB, DE was significantly reduced to 42%, 56%, 6% and 72%, respectively (Fig. 4). It was observed that the initial dye concentrations also influenced the rate of which maximum DE was achieved. Generally, 50 mg l^{-1} of initial dye concentration of CV, MV, MG and CB allowed rapid decolorization within 6 (97%), 5 (97%), 5 (98%) and 1 days (91%), respectively, while 200 mg l^{-1} required a longer duration of up to 14 days, and even then with only 7–79% DE (Fig. 5). The implications of high initial dye concentrations conforms to most observations (Radha et al., 2005; Gomashe et al., 2011; Aracagök and Cihangir, 2013), suggesting that dye toxicity may be a factor that limits decolorization activity by fungal biosorbents.

3.3.3. Influence of oxygen on TPM dye decolorization

The DE on CV, MV and CB were higher in the presence of oxygen with 82%, 82% and 86% as compared to 46%, 74% and 66% in the absence of oxygen (Fig. 6). By contrast, DE on MG was more effective in the absence of oxygen with 48% compared to 40% in aerobic conditions. In addition to higher DE, the presence of oxygen also allowed rapid decolorization activities for CB, CV and MV, achieved within 1 (91%), 7 (94%) and 7 days (97%) as compared to 13

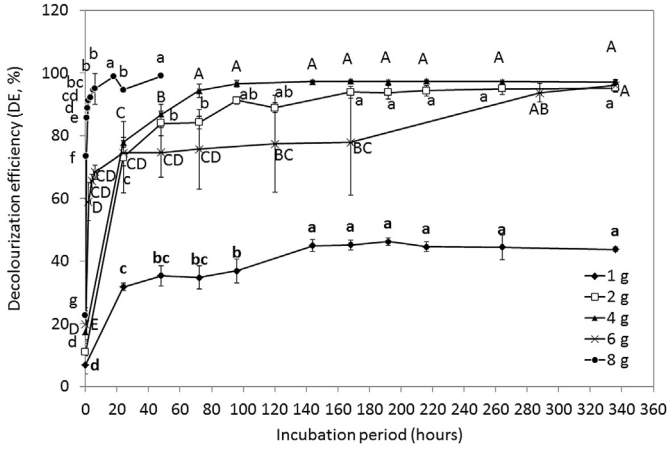

Fig. 3. Exemplary figure of the influence of biomass (1, 2,4, 6 and 8 g) of 1c3 on the decolorization activities on Crystal Violet (CV) throughout the incubation period. Means with the same letters and captions are not significantly different at Honestly Significant Difference (HSD $_{(0.05)}$). Mean comparisons are made for means with the same fonts. For example, means designated "A" compared to another mean with "A" Tukey grouping is not significantly different, while "A" compared to "B" and "C" is significantly different. Similarly, means with "a" is not significantly different compared to "a" but is for "b" and "c". Bars indicate standard deviation of mean (±SD). (Influence of biomass on decolorization activities of other dyes are provided as supplementary material).

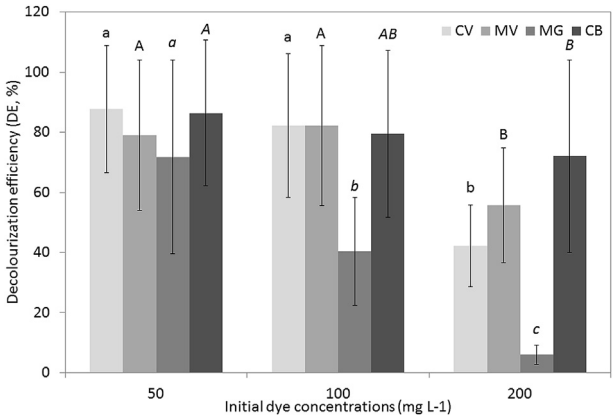

Fig. 4. Influence of initial dye concentrations on the decolorization efficiency (DE, %) of Crystal Violet (CV), Methyl Violet (MV), Malachite Green (MG) and Cotton Blue (CB) by isolate 1c3 at 30 ± 2 °C. Means with the same letters and captions within dye-groups are not significantly different at Honestly Significant Difference (HSD $_{(0.05)}$). For example, means designated "A" compared to another mean with similar "A" Tukey grouping is not significantly different, while means designated "A" compared to "a" or "*a*" or "*A*", are significantly different from one another. Bars indicate standard deviation of mean (±SD).

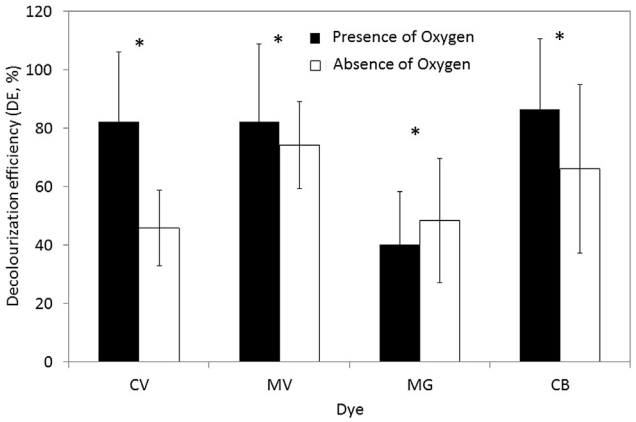

Fig. 6. Influence of oxygen on decolorization of Crystal Violet (CV), Methyl Violet (MV), Malachite Green (MG) and Cotton Blue (CB) by 1c3 at 30 ± 2 °C. "*" indicates significant difference based on paired T-test ($p < 0.05$). Bars indicate standard deviation of mean (±SD).

(89%), 14 (66%) and 14 days (98%) in the absence of oxygen, respectively (Fig. 7). This suggests that for CV, MV and CB, decolorization is primarily dependent on the oxidative reactions by key enzymes such as LiP and Lac (Shedbalkar et al., 2008; Zhuo et al., 2011; Przystaś et al., 2012). For MG, a different set of enzymes may be responsible for the decolorization activity. The higher DE achieved in the absence of oxygen (78% by day 14) compared to under aerobic conditions (52% by day 9) (Fig. 7) suggest reductive enzymes such as NADH-DCIP reductase to be responsible for decolorizing MG. Under aerobic conditions, oxygen and dye molecules compete for the reduced electron carriers, implicating enzymatic activities, leading to inferior DE for MG (Jadhav and Govindwar, 2006; Parshetti et al., 2006; Rauf and Ashraff, 2012).

3.4. Enzymatic activities

In the presence of TPM dyes, 1c3 produced more LiP and NADH-DCIP reductase as their levels were siginificantly induced compared to controls (absence of dyes in PDB) (Table 1). Lac activities on the other hand appeared to be significantly lower than in the control. This strongly suggests that biodegradation potential of 1c3 on the TPM dyes was most evidently attributed to LiP and NADH-DCIP. Although all three enzymes are crucial in degrading TPM dyes (Cha et al., 2001; Shedbalkar et al., 2008; Parshetti et al., 2011), *Coriolopsis* sp. may rely more on LiP and NADH-DCIP reductase for dye biodegradation. The role of LiP in degrading TPM dyes was contrary to (Peh et al., 2011) where Lac was the main enzyme responsible in decolorizing the azo dye RBBR (Remazol Brilliant Blue). It was also noted that NADH-DCIP was not produced in absence of TPM dyes (control), which may suggest it is specific for biodegradation of TPM dye molecules via the reductive process. NADH-DCIP levels were higher when exposed to CB and MG, while

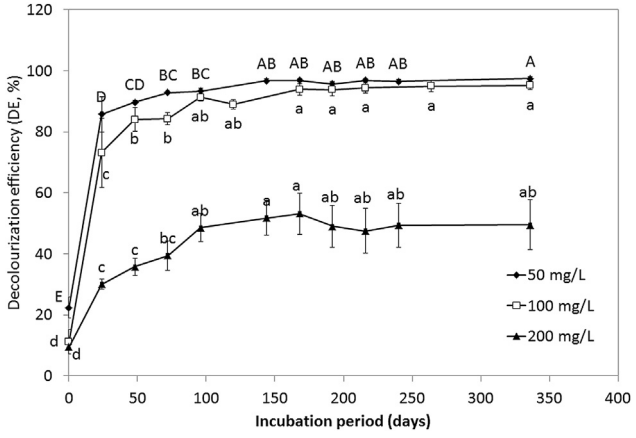

Fig. 5. Exemplary figure of the influence of initial dye concentrations on the decolorization efficiency (DE, %) of Crystal Violet (CV) by isolate 1c3 at 30 ± 2 °C throughout the incubation period. Means with the same letters and captions within dye-groups are not significantly different at Honestly Significant Difference (HSD $_{(0.05)}$). For example, means designated "A" compared to another mean with similar "A" Tukey grouping is not significantly different, while means designated "A" compared to "a" or "*a*" or "*A*", are significantly different from one another. Bars indicate standard deviation of mean (±SD). (Influence of initial dye concentrations on decolorization activities of other dyes are provided as supplementary material).

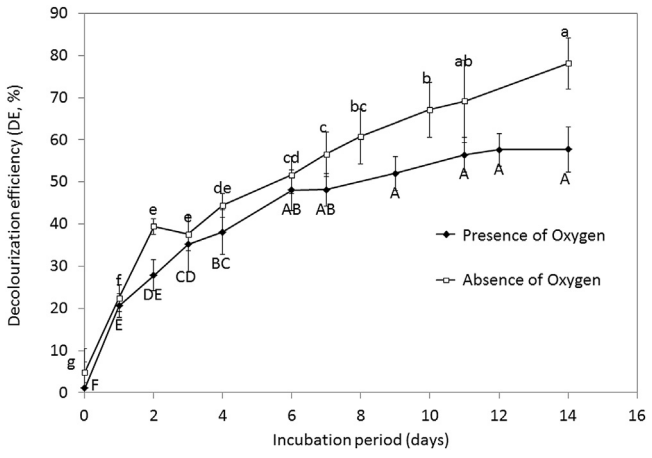

Fig. 7. Exemplary figure of the influence of oxygen on decolorization of Malachite Green (MG) by 1c3 at 30 ± 2 °C throughout the incubation period. Mean comparisons are made for means with the same fonts. For example, means designated "A" compared to another mean with "A" Tukey grouping is not significantly different, while "A" compared to "B" and "C" is significantly different. Similarly, means with "a" is not significantly different compared to "a" but is for "b" and "c". Bars indicate standard deviation of mean (±SD). (Influence of oxygen on decolorization activities of other dyes are provided as supplementary material).

Table 1
Activities of laccase (Lac), lignin peroxidase (LiP) and NADH-DCIP reductase assayed from 1c3 cultures exposed to TPM dyes compared to control (in Potato Dextrose Broth only). Data presented with standard error of mean (±SE) are mean of triplicates. "*"indicates significant difference in enzymatic levels from those in Potato Dextrose Broth based on T-test ($p < 0.05$).

Enzyme	Enzymatic activities derived from				
	PDB	CV	MV	MG	CB
Lac[a]	0.250 ± 0.001	0.007 ± 0.002*	0.094 ± 0.019*	0.176 ± 0.034*	0.051 ± 0.017*
LiP[a]	0.0002 ± 0.00001	0.0055 ± 0.0023*	0.0018 ± 0.0003*	0.0013 ± 0.0004*	0.0009 ± 0.0003*
NADH-DCIP reductase[b]	NA	642 ± 270*	464 ± 168*	1392 ± 367*	1606 ± 370*

[a] Units min^{-1} $ml^{-1.}$
[b] μmol min^{-1} ml^{-1}; NA: no activity.

the levels of LiP were highest in CV and MV dyes (Table 1). The rapid and easy decolorization of CB compared to the other dyes may be partly attributed to the high levels of NADH-DCIP produced. Lac levels were interestingly lower than in the control (PDB). Results here are however, inconclusive as only three enzymes were assayed. The possible roles of other important enzymes were not captured, especially the involvement of aminopyrine *N*-demethylase, tyrosinase, MG reductase and triphenylmethane reductase. These enzymes together with Lac, LiP and NADH-DCIP, were reportedly responsible for the degradation of CV by *Comamonas* sp. and *Delftia* sp. (Stolze et al., 2012), the degradation of CV by *Agrobacterium radiobacter* via reduction, reductive splitting and demethylation (Parshetti et al., 2011), the degradation of MV by *Aspergillus* sp. strain CB-TKL-1 via *N*-demethylation reaction, and the decolorization of MG by *Cunninghamella elegans* ATCC 36112 via reduction and N-demethylation reactions (Cha et al., 2001; Kumar et al., 2011). Nevertheless, preliminary observations on Lac, LiP and NADH-DCIP revealed that *Coriolopsis* sp. have these three enzymes and therefore can regulate biodegradation of TPM dyes.

3.5. Cell-viability under the influence of TPM dyes

Coriolopsis sp. (1c3) was observed to have high susceptibility to the presence of TPM dyes. Exposure to 50 (for CB) and 100 mg l^{-1} (MV,CV, MG) of dye concentrations for 14 days, produced only 28% viable cells from MV, while CB, MG and CV enabled recovery of only 2, 0 and 0% viable cells, respectively. It was evident that *Coriolopsis* sp. (1c3) could tolerate only 50 mg l^{-1} of dye concentrations; reaffirmed from initial dye concentration tests where % of cell viability after MV and CB decolourisation at 50 mg l^{-1} was 1940% and 349%, respectively. This concurs with other studies (Cha et al., 2001) which found that 100 mg l^{-1} of TPM dyes such as MG, could completely inhibit growth (Eichlerová et al., 2006). The use of more biomass (2, 4, 6, 8 g) compared to just 1 g for treating dyes produced varying results. There was no significant increase in the viability for 1c3 in CV and MV, but improvements were seen for 1c3 in CB and MG when 6 and 8 g of biomass were used instead. Interestingly, the presence or absence of oxygen had no significant influence on the % of viable cells used to decolourize CV, MV and MG. For CB however, presence of oxygen enhanced significantly higher % of cell viability (344%) than in absence of oxygen (0.2%). The results here offered some insights into the role of viable cells of 1c3 in removing TPM. Firstly, observations here revealed that TPM dyes are toxic to *Coriolopsis* sp. (at concentrations above 100 mg l^{-1}) and the isolate demonstrated different susceptibility to the dyes (MV > CB > CV, MG). Isolate was presumed to remove TPM dyes by biosorption and biodegradation, as decolorization occurred in dyes where non-viable (MV, CB) and viable (CV, MG) cells were detected, respectively. The production of extracellular enzymes (Lac, LiP, NADH-DCIP) by cells into the dye solution may have also contributed to biodegradation although cells may have gradually died due to toxicity. Due to the death of cells, this may have led to the longer duration required for decolorization and the possible incomplete dye degradation (Parshetti et al., 2011) as observed in this study (53–90% DE from Section 3.1).

3.6. Identification of 1c3

The isolate 1c3 was identified as *Coriolopsis* sp. based on the sequencing of its internal transcribed spacer (ITS) region of rDNA using primers ITS5/ITS4. This identitiy was provided on the basis of the nucleotide sequence having a 99% homology (E value of 0.0; 97% query coverage) with that of *Coriolopsis* sp. aff17 deposited in GenBank (accession number EU863193.2). Our sequence was deposited to NCBI and was assigned the accession number KM403574. The morphological and cultural appearances of 1c3, as well as the BLAST results and phylogenetic tree are provided as supplementary materials. The phylogenetic tree inferred that 1c3 is part of the clade consisting of *Coriolopsis polyzona voucher* strains, which has been considered in other studies as synonym to *Trametes polyzona voucher* (Justo and Hibbett, 2011). Further phylogenetic studies to determine the identity of isolate 1c3 till species level can be conducted and warrabnts the use of primers amplifying protein-coding genes such as the RNA polymerase II largest subunit (RPB1), RNA polymerase II second largest subunit (RPB2) and translation elongation factor 1-alpha (TEF1) (Justo and Hibbett, 2011). Nevertheless, this study has shown that *Coriolopsis* sp. has the potential to decolorize and possibly degrade TPM dyes.

4. Conclusions

This is the first reporting of *Coriolopsis* sp. (1c3) in decolorizing four different TPM dyes. The decolorization efficiency of this isolate was influenced by fungal biomass (minimum of 2 g), initial dye concentrations (tolerable to 50 mg l^{-1}) and requires oxygen (except for MG). Biodegradation (induction of Lac, LiP and NADH-DCIP reductase activities) played an essential role in dye decolorization, which resulted in reduced absorbance peaks for dyes. This study highlighted the potential of *Coriolopsis* sp. to be of use for the treatment of azo and TPM dyes in wastewater.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jenvman.2014.09.014.

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